Composition Studies on Tobacco. XIII. Neophytadiene Levels in Various Types and Grades[1]

Previous analytical studies have been reported on the levels of total sterols (Stedman and Rusaniwskyj, 1959a, 1960), paraffins (Stedman and Rusaniwskyj, 1959b, 1960), higher fatty acids (Swain and Stedman, 1962) and solanesol-like substances (Bilinsky and Stedman, 1962) in different tobacco types and grades. The present report is a continuation of this work and concerns the levels of the acyclic terpenoid hydrocarbon, neophytadiene (Rowland, 1957; Onishi et al, 1958; Gladding et al, 1959), in such tobaccos.

Method

Grind 12.5 g of tobacco to pass a 50 mesh screen. Extract with 250 ml of Skellysolve B[3] for 24 hours in a Soxhlet apparatus. Remove the solvent by evaporation on a steam bath under nitrogen. Dissolve the residue in 10 ml of petroleum ether. Chromatograph the solution on 50 g of activated (150° C for 16 hrs) silicic acid in a 45 x 90 mm column. Elute with petroleum ether and collect the first 200 ml of eluate. Evaporate to a residue under nitrogen on a steam bath and dissolve in 50 ml of spectral grade 2,2,4-trimethylpentane. Dilute 1 ml to 25 ml with the same solvent and determine the absorbance at 225 mμ using a 1 cm cell. Obtain the concentration of neophytadiene in the solution by reference to a standard calibration curve prepared from absorbances determined in an identical fashion on solutions containing 2, 4, 8, 12, 16 and 18 micrograms of authentic neophytadiene per ml.

The authentic neophytadiene was isolated from flue-cured tobacco and gave the following analyses: C, 86.47; H, 13.70; 2.0 double bonds per mole. $C_{20}H_{38}$ requires C, 86.25; H, 13.75; and 2.0 double bonds per mole.

Results and Discussion

Experiments in which authentic neophytadiene was added to tobacco gave recoveries of 90-95 per cent. The reproducibility was approximately ± 5 per cent for samples run on the same day; the day-to-day variation was somewhat larger. In some analyses, the ultraviolet spectrum of the residues from the petroleum ether eluates showed additional absorption in the 195-205 mμ region. Possible sources of this absorption were trace contaminants of the solvents used in the work or traces of isomeric neophytadiene produced as artifacts during chromatography (Stedman, Swain and Rusaniwskyj, 1960). The validity of the analytical procedure was not significantly affected by this absorption.

Commercial samples of unaged, flue-cured tobacco of three U. S. types, two crop years and three qualities were analyzed. The samples were graded by the organization providing the materials and were identical to those used in previous studies on higher fatty acids and solanesol-like substances. Six samples representing two crop years and three grades of a single U. S. type were run on a single day in each instance. The neophytadiene levels are given in **Table 1.** In two of the six sets of samples the neophytadiene contents of the medium and high quality grades were significantly higher than those of the lower grades. However, the lack of a conclusive quality-composition relationship is apparent.

This pattern parallels the findings in past studies in which a similar lack of a consistent relationship between leaf quality and the levels of paraffins,[4] sterols, higher fatty acids or solanesol-like substances was observed.[5]

Table 2 gives the levels of neophytadiene in samples of commercial aged or fermented tobaccos which had been previously analyzed in studies on the higher fatty acids and solanesol-like substances. Except for Burley A, all tobaccos gave neophytadiene levels lower than the range of values for the unaged bright samples. Tobaccos which had been more vigorously processed, i. e. fermented, showed neophytadiene levels not significantly less than the aged tobaccos.

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2. *Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.*
3. *Mention of a specific commercial product does not constitute endorsement by the United States Department of Agriculture over others of a similar nature.*
4. *The samples used in the paraffin and sterol investigations were U. S. grades of burley rather than the above commercial samples of bright.*
5. *However, a tendency for low grades of unaged, flue-cured leaves to have low levels of linolenic acid was noted.*

Table 2. Content of neophytadiene in aged or fermented tobaccos

Type	Neophytadiene content (%)*
Bright-Sample A	.089
Bright-Sample B	.098
Burley-Sample A	.125
Burley-Sample B	.088
Maryland-Sample A	.035
Maryland-Sample B	.040
Turkish-Sample A	.040
Turkish-Sample B	.052
Fire-cured	.084
Cigar binder (Conn.)	.084
Cigar filler (Pa.)	.055

* *Uncorrected for moisture.*

Table 1. Content of neophytadiene in unaged, flue-cured tobacco of various U. S. types, years and commercial grades

U. S. Type	Year	Neophytadiene (%) in indicated grades* Low	Medium	High
11a	1957	0.125	0.116	0.123
	1958	0.140	0.124	0.131
12	1957	0.110	0.134	0.138
	1958	0.120	0.111	0.122
13	1957	0.152	0.115	0.158
	1958	0.103	0.118	0.120

* *Uncorrected for moisture.*

Summary

An analytical method for the determination of neophytadiene was developed. The method is based on extraction of tobacco with Skellysolve B, chromatography of the extract on silicic acid and absorbance measurements at 225 mμ of the residue from the petroleum ether eluate. Commercial samples of various grades, years and U. S. types of unaged bright tobaccos and of various aged and fermented tobaccos were analyzed. No consistent relationship between grades of flue-cured leaves and neophytadiene level was observed.

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